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PRODUCTION OF HUMAN CELLS, TISSUES, AND ORGANS IN ANIMALS

Background of the Invention

This invention relates to animals that produce cells, tissues, and organs of another organism (*e.g.*, a human), and methods of generating such chimeric animals. In brief, non-functional cells, tissue(s), or organ(s) of one animal species are replaced with functional cells, tissue(s), or organ(s) from a second species by creating a chimeric animal. Potentially any cell type, tissue, or organ of an animal is amenable to this process.

Everyday, thousands of people of all ages are admitted to hospitals because of the malfunction of a vital organ and, because of a lack of organs available for transplantation, many of these people will die. Immunological incompatibility between patients and donated organs increases the possibility that, even if an organ is available, it cannot be used for a particular patient in need. Moreover, even for those patients fortunate enough to receive a donated organ, life-long administration of immunosuppressants may be required. The risk of a donated organ containing human pathogens adds another dimension to this problem.

The present invention removes both of these risks, because the donated cells, tissue(s), or organ(s) that are grown in the chimeric animal can be derived from an afflicted individual, and, thus, the transplant can be autologous.

Cloning, or nuclear transfer, is a method in which nuclear genetic material is taken from a differentiated cell of an organism and is transferred into a "reprogramming cell," which is typically an unfertilized, enucleated egg of another organism of the same species. The nucleus of this reconstructed cell dedifferentiates into a totipotent progenitor that is implanted into the uterus of a foster mother, where it develops into an organism having the same genetic makeup as the organism from which the original nuclear genetic material was derived. Additionally, cells from an organism that have been genetically modified *in vitro* can be reprogrammed to produce a clone of the original organism that contains the precise genetic modification engineered *in vitro*. Thus, cloning makes possible the generation of multiple identical organisms that contain precise genetic modifications (see, *e.g.*, Campbell *et al.*,

Nature 385:810-813, 1997: Wilmut, Scientific American, 58-63, December, 1998; Cibelli *et al.*, Science 280:1256-1258, 1998).

Summary of the Invention

In general, the invention provides animals that produce cells, tissue(s), or organ(s) of another organism (*e.g.*, a human), and methods of making such animals. Cells, tissue(s), or organ(s) produced in the animals of the invention can be taken from the animals and transplanted into patients in need of such cells, tissue(s), or organ(s).

Accordingly, in a first aspect, the invention features an animal (or precursor thereof) that contains cells, tissue(s), or organ(s) (or precursor thereof) of another organism (*e.g.*, a human), but not the corresponding cells, tissue(s), or organ(s) (or precursor thereof) that would otherwise naturally occur in the animal. The animal can be, for example, a cow, a sheep, a pig, a mouse, or a primate, such as a chimpanzee, monkey, or ape. The cells can be, *e.g.*, red blood cells, pancreatic islet cells, epithelial cells, neurons, or chondrocytes; the tissue(s) can be, *e.g.*, blood, the retina, or cartilage; and the organ(s) can be, *e.g.*, a pancreas, a heart, a liver, a kidney, intestine, a lung, or skin. Genes that can be knocked out to generate the animals of the invention include, for example, GATA-2 (blood), LMO-2 (blood), globin genes (*e.g.*, α-globin and β-globin; blood), the erythropoietin receptor gene (erythroid cells), PDX-1 (pancreas), and IPF-1 (Insulin promoter factor-1; pancreas).

In a second aspect, the invention features a method of generating an animal, as described above, which has cells, tissue(s), or organ(s) of another organism, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animal. In this method, the function of a gene that is required for development of the cells, tissue(s), or organ(s) is knocked out in a cell (*e.g.*, an embryonic, fetal, or adult fibroblast) of an animal, for example, by homologous recombination, to generate a genetically modified "cloning cell." Nuclear genetic material of the cloning cell, or a derivative thereof, is then introduced (by nuclear transfer) into a "reprogramming cell" (typically an unfertilized, enucleated egg) from another organism of the same (or different) species from which the cloning cell was obtained. Alternatively, the cloning cell is fused with the reprogramming cell. In either case the resulting reconstructed cell is stimulated to develop into an embryo. When this embryo reaches the blastocyst stage of development, "donor" embryonic stem cells of another organism (*e.g.*, a human) are introduced. The resulting blastocyst is implanted into a

pseudopregnant foster mother, where it develops into a chimeric animal that produces cells, tissue(s), or organ(s) derived from the donor embryonic stem cells, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animal. The cells, tissue(s), or organ(s) that develop from the implanted donor embryonic stem cells correspond to those cells, tissue(s), or organ(s) that are absent in the developing embryo/fetus because the gene (or genes) required for normal development and/or differentiation were knocked out in the cloning cells.

In a third aspect, the invention features a method of generating an animal, as described above, which has cells, tissue(s), or organ(s) of another organism, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animal. In this method, as described above, the function of a gene that is required for development of the cells, tissue(s), or organ(s) is knocked out in a cell (*e.g.*, an embryonic, fetal, or adult fibroblast) of an animal, for example, by homologous recombination, to generate a genetically modified cloning cell. Nuclear genetic material of the cloning cell, or a derivative thereof, is then introduced (by nuclear transfer) into a reprogramming cell (typically an unfertilized, enucleated egg) from another organism of the same (or different) species from which the cloning cell was obtained. Alternatively, the cloning cell is fused with the reprogramming cell. In either case, the resulting reconstructed cell is stimulated to develop into an embryo that is implanted into a pseudopregnant foster mother. At the appropriate developmental time, "donor stem cells" (*e.g.*, hematopoietic stem cells) of another organism (*e.g.*, a human) are surgically introduced into the developing embryo and/or fetus *in utero*, and the resulting fetus develops into a chimeric animal that produces cells, tissue(s), or organ(s) derived from the donor stem cells, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animal. The cells, tissue(s), or organ(s) that develop from the implanted donor stem cells correspond to those cells, tissue(s), or organ(s) that are absent in the developing embryo/fetus because the gene (or genes) required for normal development and/or differentiation were knocked out in the cloning cells.

In a fourth aspect, the invention features a method of generating an animal, as described above, which has cells, tissue(s), or organ(s) of another organism, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animal. In this method, as described above, the function of a gene that is required for development of the cells, tissue(s), or organ(s) is knocked out in a cell (*e.g.*, an embryonic, fetal, or adult fibroblast) of an animal, for example, by homologous

recombination, to generate a genetically modified cloning cell. Nuclear genetic material of the cloning cell, or a derivative thereof, is then introduced (by nuclear transfer) into a reprogramming cell (typically an unfertilized, enucleated egg) from another organism of the same (or different) species from which the cloning cell was obtained. Alternatively, the cloning cell is fused with the reprogramming cell. In either case, the resulting reconstructed cell is stimulated to develop into an embryo. When the embryo has developed to the morula stage (4 to 16 cell stage) the individual blastomeres are disaggregated. Chimeric morula are constructed by injecting the above disaggregated blastomeres along with disaggregated donor blastomeres of another organism (*e.g.*, a human) back into the zona pellucida or by aggregating the blastomeres of the two organisms. These reconstructed morula are implanted into a pseudopregnant foster mother, where they develop into chimeric animals that produce cells, tissue(s), or organ(s) derived from the donor blastomeres, but not the corresponding cells, tissue(s), or organ(s) that would otherwise naturally develop in the animal. The cells, tissue(s), or organ(s) that develop from the implanted donor blastomeres correspond to those cells, tissue(s), or organ(s) that are absent in the developing embryo/fetus because the gene (or genes) required for normal development and/or differentiation were knocked out in the cloning cells.

In a fifth aspect, the invention provides methods of treating patients in need of a transplant of a particular cell type, tissue, or organ, by introducing into the patient appropriate cells, a tissue, or organ produced in an animal of the invention, as described above. Also included in the invention is the use of cells, tissues, or organs produced in the invention in the treatment of disease.

The invention provides several advantages. For example, cells, tissue(s), or organ(s) produced using the methods of the invention can be grown from cells having the nuclear genetic material of a person into whom the cells, tissue(s), or organ(s) are to be ultimately transplanted, thus eliminating problems associated with adverse immunological reactions and the need for prolonged use of immunosuppressants in transplant recipients. Also, since the human cells, tissue(s), or organ(s) of the invention are produced in an animal, the cells, tissue(s), or organ(s) are free of human viruses and other pathogens, such as human immunodeficiency virus (HIV). Other features and advantages of the invention will be apparent from the following detailed description.

Detailed Description

The invention provides animals (*e.g.*, mice, cows, sheep, pigs, and primates (*e.g.*, chimpanzees, monkeys, and apes)) that produce, cells, tissue(s), or organ(s) of another organism (*e.g.*, a human), but not corresponding cells, tissue(s), or organ(s) that would otherwise naturally occur in the animals. For example, animals provided in the invention include a cow that produces human, but not bovine, red blood cells; a cow that produces human, but not bovine, blood; and a cow that produces a human, but not a bovine, pancreas. The invention also provides methods of making such animals.

The animals of the invention are made using either of three general strategies. In the first strategy, the function of a gene (or genes) necessary for the production of a particular cells, tissue(s), or organ(s) in an animal is knocked out in a cultured cell of the animal, and the nuclear genetic material of this so-called "cloning cell" (or a derivative of this cell) is introduced, by nuclear transfer, into a so-called "reprogramming cell," which is, typically, an unfertilized, enucleated egg of an animal of the same (or different) species as the animal from which the cloning cell is derived. After nuclear transfer, the resulting egg is stimulated (*e.g.*, electrically or chemically) to develop, grown into a blastocyst, into which human embryonic stem cells are introduced. The introduced human cells are out-competed in the development of all cells, tissue(s), or organ(s) of the animal, except for the cells, tissue(s), or organ(s) dependent for development on the gene (or genes) that was knocked out in the cloning cell (or its ancestor) used in the nuclear transfer. Thus, an animal that develops from this blastocyst contains normal cells, tissue(s), or organ(s) of the animal from which the cloning cell was derived, except for the cells, tissue(s), or organ(s) dependent for development on the knocked out gene (or genes); for these cells, tissue(s), or organ(s), the animal contains cells, tissue(s), or organ(s) corresponding to the organism (*e.g.*, a human) from which the embryonic stem cells that were introduced into the blastocyst were derived.

The second and third strategies differ from the first strategy only in the type of cell that is introduced into the developing animal, and in the developmental stage of this introduction. In the second strategy, the cells giving rise to the cells, tissue(s), or organ(s) to be produced in the animal (*e.g.*, human cells) are introduced later in development, for example, at 45 to 60 days gestation in the cow, and the introduced cells are further differentiated than the embryonic stem cells used in the first strategy. For example, in the generation of a cow that produces human blood, human

hematopoietic stem cells (purified from individuals or derived from embryonic stem cells *in vitro*) are surgically introduced into a 45 to 60 day old fetus *in utero* that is in the process of developing from an unfertilized, enucleated egg that had undergone nuclear transfer with a cloning cell in which a gene or genes required for hematopoiesis had been knocked out. In the third strategy, both the donor cells, giving rise to the cells, tissue(s), or organ(s) to be produced in the animal (*e.g.*, human cells), and the cloning cells are introduced early in development, at the morula stage of embryo development (4 to 16 cell embryo). For all three strategies, the donor cells that give rise to the cells, tissue(s), or organ(s) to be produced in the animal (*e.g.*, human cells) can be produced by nuclear transfer into "reprogramming cells" of the same species (*e.g.*, unfertilized, enucleated human eggs). Alternatively, the reprogramming cells can be unfertilized, enucleated eggs of a different species, for example, bovine eggs (Lanza *et al.*, Nature Medicine 5:975-977, 1999). Each of these strategies, which include many common features, are described in further detail, as follows.

Animals in which cells, tissue(s), or an organ(s) of another organism (*e.g.*, a human) can be produced include, for example, mice, cows, sheep, pigs, and primates, such as chimpanzees, monkeys, and apes. Cloning cells, thus, are selected from any of these, or other, animals for use in the invention. Preferred cells from which cloning cells are derived are those that can be grown in tissue culture and that have stable chromosomes. For example, embryonic or fetal fibroblasts can be used to make the required genetic modifications (gene(s) knockout) for use as cloning cells. Cloning cells can also be derived from more specialized or differentiated cells, such as mammary gland cells in the ewe (Campbell *et al.*, *supra*). Other cells that can be used as cloning cells for genetic modification in the invention include, without limitation, adult fibroblast cells, cumulus cells, and muscle cells. It should be noted, however, that virtually any type of embryonic, fetal, or adult cell can be used as a cloning cell in the invention. The cell that is used for cloning by nuclear transfer can be quiescent, non-quiescent, or senescent (Campbell *et al.*, *supra;* Cibelli *et al.*, *supra*; Lanza *et al.*, Science 288:665-669, 2000).

Cells of an organism (*e.g.*, a human) that can be produced in an animal according to the invention include, *e.g.*, red blood cells, pancreatic islet cells, epithelial cells, neurons, and chondrocytes; the tissue(s) can be, *e.g.*, blood, the retina, or cartilage; and the organ(s) can be, *e.g.*, a pancreas, a heart, a liver, a kidney, intestine, a lung, or skin. Genes that can be knocked out to generate the animals of

the invention include, for example, GATA-2 (blood), LMO-2 (blood), globin genes (*e.g.*, α-globin and β-globin; blood), the erythropoietin receptor gene (erythroid cells; Wu *et al.*, Cell 83(1):59-67, 1995), PDX-1 (pancreas; Offield *et al.*, Development 122(3):983-985, 1996), and IPF-1 (Insulin promoter factor-1; pancreas; Jonsson *et al.*, Nature 371(6498):606-609, 1994). In essence, any gene required for the formation of a cell type, tissue, or organ can be knocked out according to the invention to produce a genetically modified cloning cell for production of the cell type, tissue, or organ of another organism (*e.g.*, a human) in an animal of the invention.

The genetically modified cloning cells used in the invention can be generated using standard methods for knocking out genes, such as homologous recombination (see, *e.g.*, Ausubel *et al.*, eds. Current Protocols in Molecular Biology, Wiley & Sons, New York, 1989) or mismatch repair using chimeric oligonucleotides (Cole-Strauss *et al.*, Science 273(5280):1386-1389, 1996).

In most embodiments of the invention, it is not necessary to replace a knocked out gene with another functional gene. This may be desired, however, under certain circumstances. For example, if a gene product encoded by a gene to be knocked out functions as a dimeric molecule (*e.g.*, a transcription factor), it may be desirable to knock out one allele of the gene and replace this allele with a gene encoding a dominant negative mutant of the gene product. In this case, it will not be necessary to knock out the other allele, as its function will be blocked by the dominant negative mutant produced from the introduced gene. If such dominant negative mutants are not used, it is necessary to knock out both alleles of a gene. This can be accomplished several ways. If the cells have a rather long replicative lifespan, both alleles can be sequentially knocked out to produce the cloning cell desired. If the cells have a relatively short replicative lifespan, one allele can be knocked out; a fetus is generated by nuclear transfer from these heterozygous knocked out cells; a new fibroblast cell line is generated lacking a single allele; and, finally, the second allele is knocked out, generating homozygous knock out cells that are then used as the cloning cells to make chimeric animals that produce the cells, tissue(s), or an organ(s) of another organism (*e.g.*, a human) as described above. Alternatively, the lifespan of a cloning cell can be increased by introduction of a constitutively expressed telomerase gene into the cell. Additionally, cloned fibroblasts that had been grown in culture until senescence have been shown to have an extended replicative lifespan after cloning (Lanza *et al.*, *supra*). Knocking out of several genes or alleles can be carried out in such cells, without the need for intermediate cloning steps. Also, it is possible, rather than to

knock out the coding sequences of a gene, to knock out only the control elements of the gene; of course, both the coding sequences and the control elements of a gene can be knocked out.

Introduction of the genetic material of a cloning cell, generated as described above, into a reprogramming cell is carried out using cloning or nuclear transfer methods, which have been described (see, *e.g.*, Campbell *et al.*, Nature 385:810-813, 1997; Wilmut, Scientific American, 58-63, December, 1998; Cibelli *et al.*, Science 280:1256-1258, 1998; Schnieke *et al.*, Science 278:2130-2133, 1997; Brown *et al.*, Science 277:831-834, 1997). Briefly, in these methods, a nucleated cell (referred to as the "cloning cell" in this application) is fused with a recipient unfertilized, enucleated, egg (referred to as a "reprogramming cell" in this application). Alternatively, only the nuclear genetic material of the donor cell is introduced into a recipient egg. The recipient egg can be taken from an animal soon after ovulation. Such eggs are poised to begin developing once they are appropriately stimulated. The egg can be held by suction on the end of a pipette under a high-power microscope, and an extremely fine micropipette can then be used to suck the chromosomes out from the egg creating an unfertilized, enucleated reprogramming cell.

Once the nuclear genetic material of the cloning cell has been introduced into the reprogramming cell, the egg is stimulated to develop by, for example, an electrical stimulus. The stimulated egg (1) is cultured into a morula for making chimeric morula using "donor" blastomeres, (2) is cultured to blastocysts for injection of "donor" embryonic stem cells (Thomson *et al.*, Science 282:1145-1147, 1998), or (3) is implanted into the uterus of a foster mother, for the surgical transplantation of "donor" stem cells at a later developmental stage (Kennedy *et al.*, Nature 386:488-493, 1998). In each of the above scenarios, the donor cells correspond to those cells, tissue(s), or organ(s) that are absent in the developing embryo/fetus because the gene (or genes) required for normal development and/or differentiation were knocked out in the cloning cells.

In the case of a blastocyst that is implanted directly into the uterus of a foster mother, the blastocyst is allowed to develop in the mother, and stem cells, such as human hematopoietic stem cells (Kennedy *et al.*, Nature 386:488-493, 1998), are surgically introduced into the developing fetus *in utero*, for example, at 45 to 60 days of gestation in the cow. It may be desirable to introduce different types of stem cells into the developing fetus in different anatomical places and/or different developmental stages, as will be readily understood by one of skill in this art. For

example, it may be desirable to inject hematopoietic stem cells into the fetus intravenously or to introduce these cells directly into the developing fetal liver.

Once a blastocyst or fetus has been obtained that contains the desired cells from another organism (*e.g.*, a human), pregnancy is allowed to proceed. The desired cells, tissue(s), and organ(s) can be harvested at any time during further embryonic or fetal development or after birth from juvenile or adult chimeric animals.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application. All references cited herein are incorporated by reference in their entirety. Other embodiments are within the following claims.

What is claimed is: